Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Issue 3, 2012 ISSN - 0974-2441

Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITIES OF ETHANOLIC EXTRACT OF *CALOTROPIS PROCERA* FLOWERS AGAINST HUMAN PATHOGENIC STRAINS

PUSAPATI MADAN RANJIT*[1], T.SANTHIPRIYA[1], S.NAGASRI[1], Y.A.CHOWDARY[1], PASUMARTHY. N.V.GOPAL[2]

[1]NRI College of Pharmacy, Department of Pharmaceutical Biotechnology, Pothavarappadu (V) Agiripalli (M), Krishna District, A.P, India,[2]Hindu College of Pharmacy, Department of Pharmaceutical Biotechnology, Amaravathi Road, Guntur, A.P, India ,
E-mail: madanranjit@gmail.com

Received: 21 March 2012, Revised and Accepted: 18 May 2012

ABSTRACT

Ethanolic extract of flowers of *Calotropis procera* R. Br. (Asclepiadaceae) shows antibacterial action against four Gram positive micro-organisms, (*Staphylococcus aureus, Bacillus subtilis, Bacillus pumilis,* and *Micrococcus lutes* and Gram Negative micro-organism (*Escherichia coli Pseudomonas aeruginosa,* and *Proteus vulgaris*). The antibacterial action of extract is compared with a standard drug (rifampicin, 50µg/ml) at various concentrations (5, 10, 20, 30 and 50 µg/ml).Before that preliminary phytochemical screening was performed by using a standard chemical test and antibacterial action screened by using Cylinder-plate assay method. Phytochemical analysis of the flowers extract showed the presence of tannins, steroids, saponins and flavonoids while alkaloids are absent. Phytochemical investigations explore active constituents which are very significant in drug development. The study revealed a notable antibacterial inhibitory activity of ethanolic extract of the flowers.

Keywords: *Calotropis procera,* Preliminary phytochemical screening, Cylinder-plate assay method.

INTRODUCTION

Calotropis procera R. Br. (Asclepiadaceae) is a plant widely distributed in Asia, Africa, and Northeast of Brazil. The plant is popularly known because it produces large quantity of latex, which is easily collected from its green parts, when the plant is wounded (Larhsini et al. 1997, Haque et al., 2000). The milky latex is locally applied in the treatment of cutaneous diseases such as ringworm, syphilitic sores and leprosy (Kew, 1985). In Ayurveda: the Indian system of medicine, this plant is reported for the treatment of several infectious diseases including purulent wound infections (Sharangdhar Samhita, 1964, Charaka Samhita, 1952, Sushruta Samhita, 1955). The plant has been widely used in the traditional system of medicine for the treatment of various ailments. It has been used as a purgative, antihelmintic, digestive, stomachic, emetic, expectorant, sedative, an antidote for snake poisoning and for the treatment of ulcers, tumors, leprosy, asthma, boils, dysentery, eczema, piles, diseases of liver, and spleen disorders (Kirtikar and Basu, 1935, Nadkarni and Nadkarni, 1960). This study showed preliminary phytochemical screening and antibacterial activities of *Calotropis procera* flowers extract against human pathogenic strains.

MATERIALS AND METHODS

Plant Material

The flowers are collected in month of September-2010 from out fields of Agiripalli Mandal, Krishna district, identified and herbarium specimen was deposited in the department of Pharmacognosy with specimen No.NRI/COL/P.COG/1/PF (Flowers). It was air dried under shade at ambient temperatures and grounded to small. The plant was first identified at the field using standard keys and descriptions (Gill, 1987).

Preparation of extracts

The shade-dried powder of flowers was subjected to extraction in soxhlet extractor with 70% EtOH for 70 hours (extract yield: 9%) and extract is collected. The collected extract is evaporated to dryness and stored at 4 ◦C until used.

PHYTOCHEMICAL SCREENING

Test for reducing sugars

One gram of the ethanolic extract was weighed and placed into a test tube. This was diluted using 10 ml of de-ionised distilled water. This was followed by the addition of Fehling's solution. The mixture warmed to 40ºC in water bath. Development of brick-red precipitate at the bottom of the test tube was indicative of the presence of a reducing sugar (Brain and Turner, 1975).

Test for Protein

Millons test, Crude ethanolic extract was mixed with 2 ml of Millons reagent (mercuric nitrate in nitric acid containing traces of nitrous acid), white precipitate appeared, which turned red upon gentle heating. Ninhydrin test, Crude extract when boiled with 0.2 % solution of Ninhydrin, violet color appeared, indicates presence of amino acids and protein (Debela, 2002).

Test for Fat

Stain test, the small quantity of crude ethanolic extract was pressed between two filter papers; the stain on 1st filter paper indicated the presence of fixed oils. Saponification test, In small quantity of crude extract few drop of 0.5N of alcoholic potassium hydroxide were added to which a drop of phenolphthalein was added separately and heated in a water bath for 1 hour. The formation of soap indicated the presence of fixed oils and fats (Debela, 2002).

Test for resins

Two grams of the ethanolic extract was dissolved in 10ml of acetic anhydride. A drop of concentrated sulphuric acid was added. Appearance of purple colour, which rapidly changed to violet, was indicative of the presence of resins. Same procedure was repeated using the aqueous extract of the plant material (Cuilel, 1994).

Test for tannins

Two grams of the ethanolic extract was weighed and placed in a test tube. Two drops of 5% ferric chloride solution was then added. The appearance of a dark green color was indicative of the presence of tannins. The same procedure was repeated using the ethanolic extract (Cuilel, 1994).

Test for steroids

One gram of the ethanolic extract was weighed and placed in a test tube. This was dissolved in 2 ml of acetic anhydride, followed by the addition of 4 drops of chloroform. Two drops of concentrated sulphuric acid were then added by means of a pipette at the side of the test tube. The development of a brownish ring at the interface of the two liquids and the appearance of violet colour in the supernatant layer were indicative of the presence of steroid

glycosides. Same procedure was repeated using the aqueous extract (Cuilel, 1994).

Test for flavonoids

Two grams of the ethanolic extract was weighed, placed in a test tube, followed by the addition of 10 ml of DMSO. The mixture was heated, followed by the addition of magnesium metal and 6 drops of concentrated hydrochloric acid. The appearance of red colour was indicative of the presence of flavonoids. Same procedure was repeated using aqueous extract (Sofowora, 1993).

Test for alkaloids

One gram each of the ethanolic extract was weighed and placed into two separate test tubes. To the first test tube, 2-3 drops of Dragendoff's reagent was added while 2-3 drops of Meyer's reagent were addedto the second test tube. The development of an orange-red precipitate (turbidity) in the first test tube (with Dragendoff's reagent) or white precipitate (turbidity) in the second test tube (with Meyer's reagent) was indicative of the presence of alkaloids (Cuilel, 1994).

Test for saponins

Five grams of the ethanolic extract was weighed and placed in a test tube. This was followed by the addition of 5 ml de-ionised distilled water. The content was vigorously shaken. The appearance of a persistent froth that lasted for 15 minutes was indicative of the presence of saponins (Brain and Turner, 1975). Table 1 shows the presence of phytochemical characteristics of ethanolic extract flowers.

ANTIBACTERIAL ACTIVITY

Test organisms

The selected NCIM (National Collection of Industrial Micro-organisms) type bacterial stains were provided by Department of Pharmaceutical Biotechnology, Hindu College of Pharmacy, Amaravathi Road, Guntur**.** The selected micro-organism was listed below: Four Gram positive micro-organisms (*Staphylococcus aureus* NCIM 2079, *Bacillus subtilis NCIM 2063, Bacillus pumilis NCIM 2327,* and *Micrococcus lutes* NCIM 2871) and Gram Negative micro-organism (*Escherichia coli* NCIM 2067, *Pseudomonas aeruginosa* NCIM 2037, *and Proteus vulgaris* NCIM, 2027).

Preparation of inoculums

The *in vitro* screening of antibacterial activity was carried out using Cylinder-plate assay method. For antibacterial activity, the inoculums or microbial suspension is prepared according to the procedure given in the I.P (Indian pharmacopeia-2010).The test organism (one loop full) were seeded to the nutrient agar (HIMEDIA) at temperature between 40° and 50° and immediately pour the inoculated medium into the Petri plate (8 Inch) to give a depth of 3 to 4 mm and allowed to solidify and punched with a sterile cork borer (6.0 mm diameter) to make open cavities. Each plate had maximum seven cavities with appropriate distances.

Preparation of test and standard solutions

The stock solution of test sample was prepared by dissolving the dried ethanolic extracts of flowers of *Calotropis procera* at concentration of 5,10,20,30 and 50 µg/ml in dimethylsulphoxide (DMSO) respectively. The stock solution of reference standards (Rifampicin) was prepared at a concentration of 1mg/ml in DMSO. The 50µg/ml rifampicin was used as positive control and 0.05 ml of DMSO was used as negative control. Antimicrobial activity was screened by adding 0.05 ml both test and standard solution to each cavity of the plate (set-1) by using micropipette. This method is performed for two more sets (Set-2, Set-3). All the plates were kept for 1 to 4 hours at room temperature and incubate them for about 18 hours at the temperature. After incubation the bacterial inhibition zone were measured in diameter with cavity from the average of three plates.

RESULTS AND DISCUSSION

Many naturally-occurring compounds found in plants have been shown to possess antimicrobial functions and could thus serve as a source of traditional drugs (Kim et al., 1995).**Table:2** shows the antibacterial activities of ethanolic extract of flowers against various microbial strains, with respect to various concentrations (µg/ml).The inhibition zones of test concentrations were compared with the standard concentration of rifampicin (50µg/ml) by using Tukey-Kramer multiple comparisons test. **Fig**: 1, 2, 3, 4, 5, 6 and 7 shows that significant results ($p<0.001$ to 0.05) when compared the test concentrations versus standard drug. In the case of *Staphylococcus aureus* NCIM 2079, at test concentration (50µg/ml) versus standard drug (50µg/ml) showed insignificant ($p>0.05$). The antibacterial activities observed could be due to the presence of secondary metabolites. Some other reports are also reported that, various parts of this pant shows that antimicrobial activities (Kawo et al., 2009, Kareem, 2008, Bhaskar, 2000). Based the preliminary phytochemical screening of ethanolic extract of flowers of this plant, if properly screened by using additional solvents, could yield new antimicrobial drugs. Further research is therefore recommended to isolate, purify and characterize these chemical constituents.

CONCLUSION

As per our knowledge Ethanolic extract of flowers of *Calotropis procera* showed the antibacterial action in dose dependent on different pathogenic stains. Further studies are needed for confirmation of antibacterial action by isolating pure chemical constituents and also indentify which compound is responsible for antibacterial action of *Calotropis procera* flowers.

Table 1: Phytochemical characteristics of ethanolic extract of *Calotropis procera* flowers

Ingredient	Ethanolic (70%) extract
Reducing sugar	+
Proteins	+
Fats	-
Resins	+
Tannins	+
Steroids	+
Flavonoids	+
Alkaloids	-
Saponins	+

Key: + = Present; - = Absent.

Table 2: Shows the inhibition zone diameters of various concentration of ethanolic extract versus standard concentration of rifampicin

Type of strain	Zone diameters (mm) with respect to Conc. of the ethanolic extract						Conc of Rifampicin
	5µg/ml	10µg/ml	20µg/ml	30µg/ml	50µg/ml	Control	50µg/ml
B.S	10.3 ± 0.2236	12.3 ± 0.2236	13.3 ± 0.2236	14.3 ± 0.2236	15.3 ± 0.5143	7	17.3 ± 0.6708
B.P	12 ± 0.4472	13.3 ± 0.2236	14.3 ± 0.2236	15 ± 0.2236	15.6 ± 0.6708	7	19.6 ± 0.6708
M.L	9.6 ± 0.2236	11.6 ± 0.6708	13.3 ± 0.2236	14.6 ± 0.4919	15.6 ± 0.4919	7	20 ± 0.7603
P.A	10.3 ± 0.5000	11 ± 1.000	13.3 ± 0.5000	14.6 ± 1.100	16 ± 1.000	7	21.3 ± 1.500
S.A	10.3 ± 0.2236	11.3 ± 0.4472	11.6 ± 0.2236	13 ± 0.000	18.3 ± 0.2236	7	19 ± 0.4472
E.C	10.3 ± 0.6708	12.6 ± 0.4919	14.6 ± 0.4919	14.6 ± 0.2236	17.3 ± 0.6708	7	20.3 ± 1.029
P.V	10.6 ± 0.2236	12.3 ± 0.6708	14 ± 0.4472	16 ± 0.4472	18.3 ± 0.6708	7	23 ± 0.4472

The valves of each concentration of mean ± standard error by mean (S.E.MS) of three replicates, Standard drug rifampicin 50µg/ml, and Ethanolic extract of flower of *C.Procerea* concentration (5µg/ml, 10µg/ml, 20µg/ml, 30µg/ml, and 50µg/ml).Control: Dimethylsulphoxide (DMSO). B.S: *Bacillus subtilis* NCIM 2063,B.P: *Bacillus pumilis* NCIM 2327,M.L: *Micrococcus lutes* NCIM 2871,P.A: *Pseudomonas aeruginosa* NCIM 2037,S.A: *Staphylococcus aureus* NCIM 2079,E.C*: Escherichia coli* NCIM 2067,P.V: *Proteus vulgaris* NCIM 2027

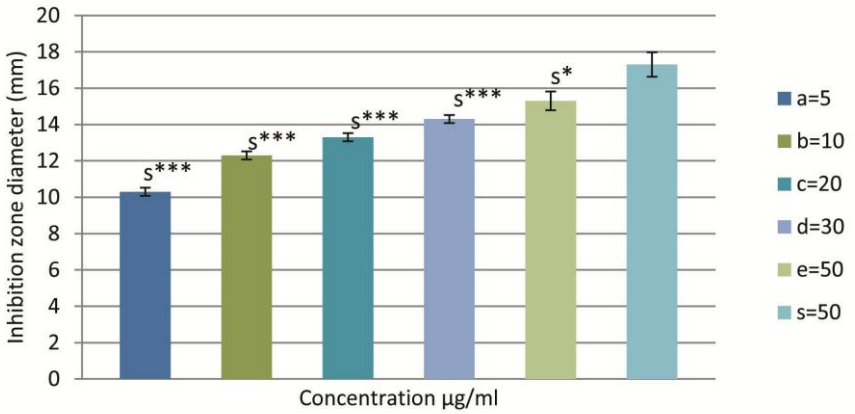

Fig 1:Ethanolic extract of flower of *C.Procerea* shows concentration (µg/ml) versus inhibition zone diameter (mm) against *Bacillus subtilis NCIM 2063*.Tukey-Kramer Multiple Comparisons test shows the comparison the test concentration a=5µg/ml, b=10µg/ml, c=20µg/ml, d=30µg/ml, and e=50µg/ml versus s=50µg/ml concentration of standard. The test concentration of a b, c, d, are $p < 0.001$ and e is $p < 0.05$.

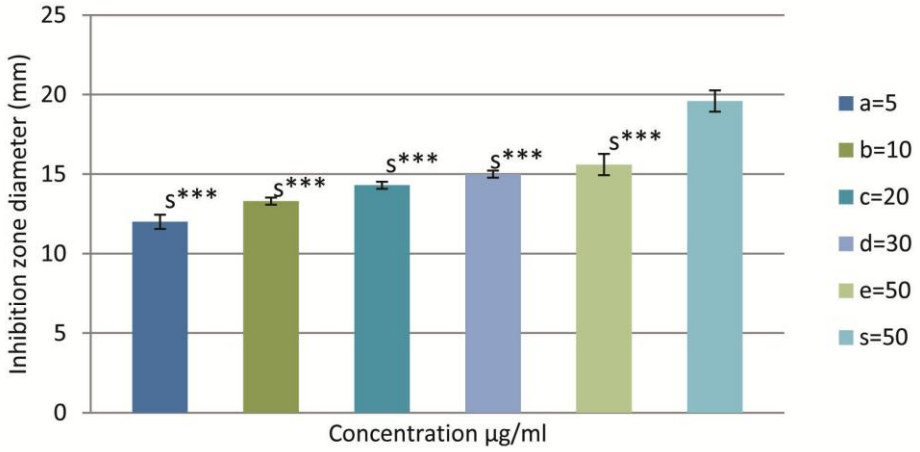

Fig 2:Ethanolic extract of flower of *C.Procerea* shows concentration (µg/ml) versus inhibition zone diameter (mm) against *Bacillus pumilis NCIM 2327*.Tukey-Kramer Multiple Comparisons test shows the comparison of test concentrations, a=5µg/ml, b=10µg/ml, c=20µg/ml, d=30µg/ml, and e=50µg/ml versus s=50µg/ml concentration of standard. The test concentration of a b, c, d, and e are $p < 0.001$.

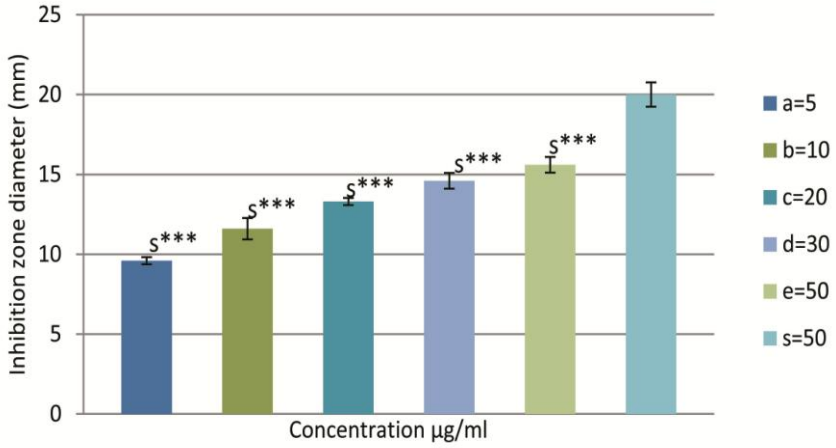

Fig 3: Ethanolic extract of flower of *C.Procerea* shows concentration (µg/ml) versus inhibition zone diameter (mm) against *Micrococcus lutes* NCIM 2871.Tukey-Kramer Multiple Comparisons test shows the comparison of test concentration, a=5µg/ml, b=10µg/ml, c=20µg/ml, 30µg/ml, and e=50µg/ml versus s=50µg/ml concentration of standard. The test concentration of a b, c, d, and e are $p<0.001$.

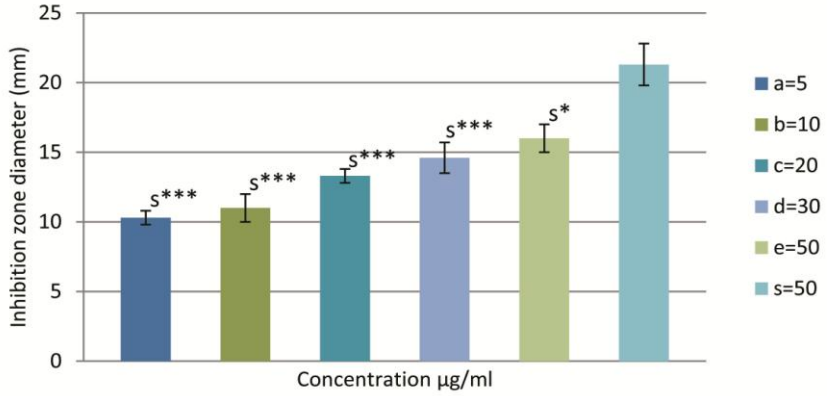

Fig 4:Ethanolic extract of flower of *C.Procerea* shows concentration (µg/ml) versus inhibition zone diameter (mm) against *Pseudomonas aeruginosa* NCIM 2037.Tukey-Kramer Multiple Comparisons test shows the comparison of test concentration, a=5µg/ml, b=10µg/ml, c=20µg/ml, d=30µg/ml, and e=50µg/ml versus s=50µg/ml concentration of standard. The test concentration of a b, c, d, are $p<0.001$ and e is $p<0.05$.

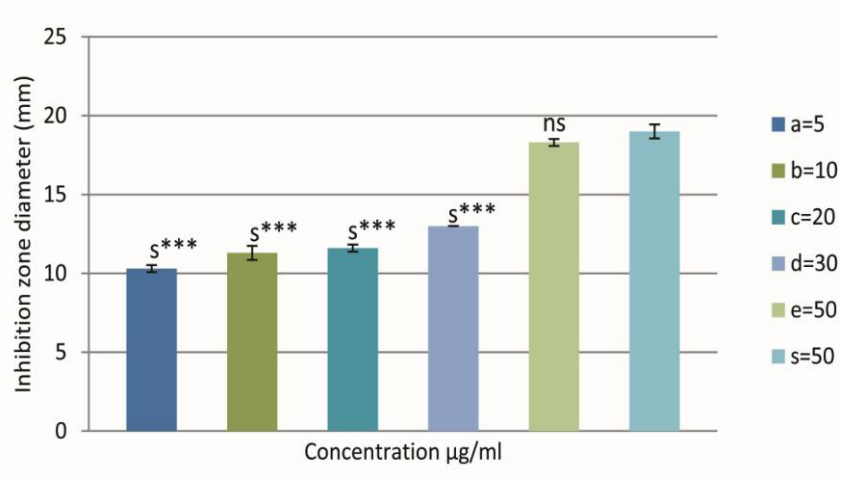

Fig 5:Ethanolic extract of flower of *C.Procerea* shows concentration (µg/ml) versus inhibition zone diameter (mm) against *Staphylococcus aureus* NCIM 2079.Tukey-Kramer Multiple Comparisons test shows the comparison of test concentration, a=5µg/ml, b=10µg/ml, c=20µg/ml, d=30µg/ml, and e=50µg/ml versus s=50µg/ml concentration of standard. The test concentration of a b, c, d, are $p<0.001$ and e is $p>0.05$.

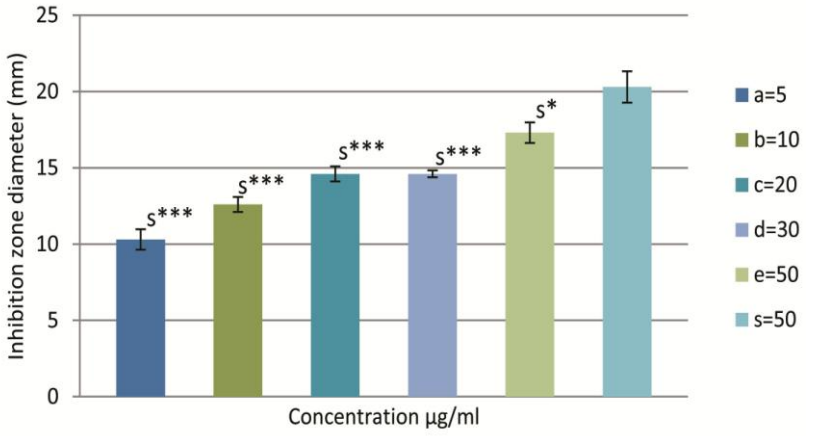

Fig 6:Ethanolic extract of flower of *C.Procerea* shows concentration (µg/ml) versus inhibition zone diameter (mm) against *Escherichia coli* NCIM 2067.Tukey-Kramer Multiple Comparisons test shows the comparison of test concentration, a=5µg/ml, b=10µg/ml, c=20µg/ml, d=30µg/ml, and e=50µg/ml versus s=50µg/ml concentration of standard. The test concentration of a b, c, d, are $p<0.001$ and e is $p<0.05$.

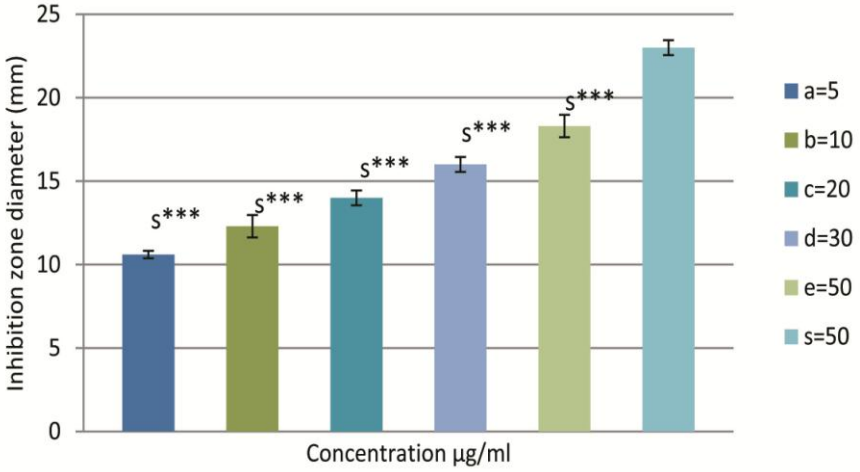

Fig 7: Ethanolic extract of flower of *C.Procerea* shows concentration (µg/ml) versus inhibition zone diameter (mm) against *Proteus vulgaris* NCIM 2027.Tukey-Kramer Multiple Comparisons test shows the comparison of test concentration, a=5µg/ml, b=10µg/ml, c=20µg/ml, d=30µg/ml, and e=50µg/ml versus s=50µg/ml concentration of standard. The test concentration of a b, c, d, and e are $p< 0.001$.

ACKNOWLEDGEMENTS

The authors are thankful to the management and principal of NRI College of Pharmacy, Pothavarappadu, Agiripalli mandal, Krishna Dist, A.P, India, for providing the research facilities for carrying out this research work.

REFERENCES

1. Larhsini M, Bousaid M, Lazrek HB, Jana M, Amarouch H, 1997, Evaluation of antifungal and molluscicidal properties of extracts of Calotropis procera. Fitoterapia 68: 371-373.
2. Haque MA, Nakakita H, Ikenaga H, Sota N, 2000, Development-inhibiting activity of some tropical plants against Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae). J Stored Prod Res 36: 281-87.
3. Kew F. 1985, The useful plants of West Tropical Africa, Vol. (1). Families A – D Edition 2 (edurkill, HM.), Royal Botanical Gardens. Pp.219-222.
4. Sushruta Samhita, translated by Govindgi K, 1965, Published by Sastu Sahitya, Near Badthra, Ahmedabad, Gujarat, India, 1964, Vide Vaidya BG, Nighantu Aadarsha, Vol.2, pp.683-690.
5. Charaka Samhita, 1965, Translated by Shastri Gyrijashankar Mayashankar. Published by Sastu Sahitya, Near Badthra, Ahmedabad, 1st Edn, 1952, Vide vaidya BG, Nighantu Aadarsha, vol.2,pp.683-690.
6. Sharangdhar Samhita, 1965, Translated by Rasioklal Jethalal Parikha, published by Sastu Sahitya, Near badthra, Ahmedabad, 1st Edn, Vide Vaidya BG, Nighantu Aadarsha, Vol.2 pp.683-690.
7. Kirtikar KR Basu BD, 1935, Indian Medicinal Plants, M/S Bishen Singh Mahendra Pal Singh, Dehradun, 2nd Edition. Pp.1606-1607.
8. Nadkarni AK. Nadkarni KM, 1960, Indian Materia Medica, Popular Book Depot, Bombay. pp. 69-71.
9. Gill LS, 1987, Taxonomy of flowering plants. African-Feb publishers limited, Book house, Onitsha, Nigeria, Pp.1-200.
10. Brain KR. and Tuner TD, 1975, The practical evaluation of phytopharmaceuticals. Wright Scientectica Publishers, Bristol. Pp 57-58.
11. Debela A, 2002, Manual for Phytochemical Screening of Medicinal Plants. Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia, 35-47.
12. Cuilel I, 1994, Methodology for the analysis of vegetables and drugs. Chemical Industry Division,NNIDO Romania, Pp24-67.
13. Sofowora A, 1993, Medicinal plants and traditional medicine in Africa. Spectrum books limited, Ibadan, Nigeria. pp 220-224.
14. Brain KR. and Tuner TD, 1975, The practical evaluation of phytopharmaceuticals.Wright Scientectica Publishers, Bristol. Pp57-58.
15. Kim J, Marshal MR. and Wei C. 1995. Antibacterial activity of some essential oil components against five food-borne pathogens. Journal of Agriculture and Food Chemistry 43:2839-2845.
16. Kawo AH, Mustapha A, Abdullahi BA, Rogo LD, Gaiya ZA and Kumurya AS. 2009. Phytochemical properties and antibacterial activities of the leaf and latex extracts of calotropis procera (ait.f.) ait.f. Bayero Journal of Pure and Applied Sciences, 2(1): 34 -40.
17. Kareem SO, Akpan I., Ojo OP, 2008, Antimicrobial Activities of *Calotropis procera* onSelected Pathogenic Microorganisms. African Journal of Biomedical Research 11, 105 –110.
18. Bhaskar VH, 2000, Antimicrobial Activity of *Calotropis procera* Seeds. Asian journal of chemistry 21 (7), 5788-5790.